A COMPARATIVE STUDY IN METHODS OF PLANKTON MEASUREMENT.

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ASSISTED BY H. W. GRAYBILL AND OTHERS.

WITH PLATES XV, XVI AND XVII.

INTRODUCTION.

During the course of a biological investigation on Lake Michigan in the Traverse Bay region, carried out under the auspices of the Michigan Fish Commission, a series of about one hundred plankton hauls was made in Lake Michigan and in the adjoining Round and Pine Lakes. These were taken between August 11 and 28, 1894, and are more fully described and located in a previous paper (Ward, 96b). A few of these hauls were measured within a short time after being taken, and some preliminary experiments on the details of the method to be used were made between November, 1894, and February, 1895. These were not employed in the subsequent discussion of the results of the work, for frequent interruptions made it difficult for me to follow a uniform system of measurement and in February, 1895, I secured the cooperation of one of my assistants, Miss Anna Fossler, who daily at a given time set some hauls each in a settling tube, from which the amount was read off after a lapse of exactly twenty-four hours. All of the readings in this series were verified by me at the time of recording them and were used in the papers (96-96b) on the results of the work. Consequently this series is designated as the original one (0, Pl. XV-XVII).

It was noticed, however, that the amounts obtained did not agree exactly with those recorded for certain hauls in the pre-

liminary experiments and more than a year and a half later Miss Fossler at my request repeated the measurements in the same tubes and as nearly as possible in the same manner as before. This second set of gravity measurements was made between December 26, 1896, and January 16, 1897, and is designated as the first supplementary set (1, Pls. XV-XVII). During some work on the Great Lakes the following year I became impressed with the advisability of careful, comparative measurements on a series of plankton hauls as a means of determining the various errors and variations to which the method was subject; and at my request Miss Fossler again repeated the measurements. This, the third set of gravity measurements, was made between August 19 and September 7, 1898, and is designated as the second supplementary series (2, Pls. XV-XVII). It was made thus more than three years after the original series, and was the last made by this method. It should further be noted that all three sets of gravity measurements were made not only in the same manner and with the same apparatus, but also by the same person. Any differences which may be found will therefore indicate those variations normal to the method.

It seemed also of importance to have for comparison a series of measurements of the same plankton hauls by means of the centrifuge which has been in use in a number of places in such work.* And in April, 1899, my assistant, Mr. J. A. Britton, measured the set of plankton hauls in a Bausch & Lomb Urine Centrifuge. The plankton was settled in the ordinary sedimentation tubes, being kept in revolution one minute, during which time the crank was given eighty complete revolutions. This first series of centrifuge measurements is designated as the third supplementary set (3, Pls. XV-XVII). The fourth sup-

* Juday (97) was apparently the first to publish an account of the use of the centrifuge for this purpose. Both Dr. Kofoid and I had, however, experimented independently for more than a year before that and had written to various investigators regarding the advantages of such an instrument.

plementary set (4, Pls. XV-XVII), which is the second series of centrifuge measurements, was made in January, 1900, by Mr. H. W. Graybill, who became much interested in the problem and performed all the extended and tedious mathematical operations necessary to reduce the volumes obtained to common terms with those used in the original paper (Ward, 96b). He also made the plates accompanying this paper and participated in the general discussion of its various points towards which he contributed items of value. While we were discussing the comparative results of the first and second sets of centrifuge measurements during the interval of the delayed appearance of this paper, Mr. Graybill suggested that as three sets had been made by the gravimetric method, a just comparison could only be made were there three sets of centrifuge measurements as well, since the chance of departure from the mean is evidently less in a double than in a triple set. He made then (April, 1900) a third set of centrifuge measurements which was added to the charts (5, Pls. XV-XVII) and text. The results of this set abundantly justified his prediction for after all possible care to make the measurements uniform throughout, combined with some skill in the use of the method, it was found that the measurements of the third set made by the centrifuge fell more frequently outside than within the limits of the first two sets made by the same instrument, as is distinctly shown on the plates. In all the centrifuge measurements the same instrument was employed and the same method followed in detail. Some experiments were tried in varying the conditions, particularly of time, but extended tests could not be made.

It should be noted further that all records were made and entered without any knowledge of former results or comparison with them, and the time interval was sufficient to preclude the possibility of remembering previous figures so that the estimation of the amount was made in an entirely unprejudiced manner. No haul was omitted because it seemed to "spoil the average" and the few gaps in the record are due to the accidental destruction of a haul, or to its utilization at an earlier date for other purposes (Ward, 96a).

While the senior author is alone responsible for the text of the paper, his sincere thanks are due to those who have co-operated so kindly in the accumulation and elaboration of the data on which it rests. Mr. Graybill has also participated in the discussion which has reduced the paper to its final form.

ERRORS IN PLANKTON DETERMINATION.

Quantitative determinations of the amount of floating organisms in fresh water have been made in various parts of the world and the methods and apparatus employed therein have been noticeably different. These investigations were summarized in a previous paper (Ward, 99) and need not be considered further here. Several investigators have called attention to various points of weakness in the methods and to the difficulty or impossibility of comparing results obtained under such radically different conditions. But so far as I know no one has yet endeavored to ascertain the limit of accuracy in these determinations, and the means by which different sets of observations may be reduced to common terms for comparison. It is my purpose to offer here some data towards the solution of this important question.

The accuracy of a plankton determination is of course dependent upon the accuracy of the various stages in the process so that the latter must necessarily be subject to analysis at first. One may readily distinguish four chief stages in the method: a) the process by which a certain quantity of plankton is obtained; b) the treatment involved in the permanent preservation of the quantity obtained; c) the determination of the volume obtained; and d) the enumeration or estimation of the individuals in this volume. A number of subordinate steps may be distinguished under each chief division of the process noted, as will appear later.

An extended discussion of the first question: Does the method employed in obtaining the plankton actually catch all the planktonts, has been given by Kofoid (97a) who emphasizes the loss incurred by the use of the vertical net and the advan-

tages accruing from the employment of a plankton pump. The disadvantages of the latter in cost, weight and lack of wide applicability have been urged by Reighard (98) and Fuhrmann (99). The use of the vertical net will evidently be more satisfactory when its actual efficiency as a catching apparatus has been determined by precise and full experimentation. Now it can only be said that the loss is real, considerable in some groups, and though perhaps numerically large, yet probably volumetrically small. Much more important it is to determine whether this loss is constant or variable, and if the latter under what conditions or in what way it varies. These questions have been discussed at length by various authors, but as yet no data are at hand which can be said to settle the matter definitely. For not only do opinions differ widely as to the possibility of determining the coefficient in a given net and as to its constancy under various circumstances, but there is also no basis for comparison between the efficiency of different nets and consequently no idea can be given as to the relative meaning of results obtained by different observers. They stand absolutely isolated and unrelated. It is important to establish the actual variation in a given net, and then to standardize the net by comparison with some other net having a known catching value or with some normal unit to be chosen. This is evidently necessary before it will be possible to compare results obtained by various investigators in different parts of the world.

On the second question as to whether any part of the catch is lost in subsequent manipulation, no precise calculations have been published. Careful observations made at Charlevoix by Dr. Kofoid at my suggestion failed to reveal any measurable loss of material during preservation of the hauls made there (Ward, 96) and improvements of the method introduced since then (Kofoid, 97) by which the plankton is transferred directly from the bucket of the net to the bottle in which it is preserved, tend to reduce to the minimum the loss of plankton during the process of preservation. There is, then, I believe reason for disregarding this possible error as inconsiderable in amount.

On the question of the determination of the plankton volume it may be said that the plankton hauls taken in fresh water have generally been measured volumetrically either by settling in a graduated tube or by the use of a centrifuge. Although the probability of variation in the first has been marked by nearly every author who has employed the method, no one has yet so far as I know given data to fix the amount of such variation under different conditions or to render possible a comparison of amounts obtained by gravitation with others measured in the centrifuge. The extensive series of measurements referred to in the introduction throws some light on this question in its various aspects.

First may be discussed the evidence as to variations in the measurement of plankton by settling, i. e., in the gravitation method. No hauls were measured absolutely fresh but a set of seven were permitted to settle in graduated tubes within 46 hours of the time they were taken. So far as these are concerned four were larger and three smaller than any subsequent measurements of the same hauls made by the gravitation method.*

In the first measurements of plankton hauls made by the gravity method during the winter following their being taken it was noticed that circumstances exercised a considerable influence on the result. The method followed involved the removal of a tube from the rack where it had stood, the notation of the volume and the return of the tube with all possible care to its place in the rack. If a reading of the amount was made after twenty-four hours and the tube returned to the rack where it had been standing, a second reading taken at a nominal interval thereafter would differ from the first. The difference

* At the time these were first measured I had not noted the effect of various factors mentioned below, so that there is no evidence that the measurements were made under identical circumstances. The absence of noteworthy difference merely favors the presumption that newly killed plankton does not behave differently from that which has been killed a much longer time. It should be said that the differences between the measurements of these hauls are greater in both directions than those recorded in other cases and greater than those listed for these same hauls later, due probably to lack of experience in the use of the method.

was naturally always a loss from the volume first noted and the figures given in the table following denote the number of cases for each error observed.

Loss at second reading	Less than 1 per cent.	1 to 5 per ct.	6 to 10 per ct.	11 to 15 per ct.	16 to 20 per ct.	Average per ct.
After an interval of 5 minutes...	1	3	5	0	0	5.5
10 " ...	0	0	2	4	0	10.0
15 " ...	1	10	8	4	1	7.0
20 " ...	1	1	1	1	0	5.5
40 " ...	0	0	1	2	0	14.0

A third reading still later than the above gave the following results. The time is recorded from the first reading which was in all cases twenty-four hours after the plankton tube was set aside to settle.

Af er an interval of	5 per ct. or less	6 to 10 per ct.	11 to 15 per ct.	16 to 20 per ct.	21 to 25 per ct.	Average loss per ct.
2 hours....	0	0	0	0	3	22.0
2 days.....	1	2	1	0	0	9.0
3 "	2	2	4	3	1	12.5
4 "	1	1	2	6	0	15.4
5 "	0	0	0	1	0	16.0
6 "	0	0	5	5	0	15.0
33 "	0	3	1	0	0	9.5

The amount of reduction in any given plankton volume, measured by the gravity method, depends thus only very generally on the lapse of time, but is affected much more prominently by the disturbances to which the tube is subjected during the settling period. This is clearly shown by a series of measurements on the same plankton haul, left standing for some time and measured at varying intervals, at each of which the tube was moved as above.

	20 min.	4 days	5 days	6 days	6¼ days
Loss per cent. in volume.....	9	15	16	18	20

	15 min.	21 days	22 days	43 days
Loss per cent. in volume...	3	4	9	11
	3	6	7	8

Numerous experiments were made with the plankton hauls to determine the difference in volume dependent upon length

of time when the settling progressed undisturbed. Thus one series of tubes was left twenty-four hours and after the volume was recorded, thoroughly shaken and permitted to stand undisturbed either twenty-four or forty-eight hours longer with the following results based on comparison with original measurements of same.

Loss	Less than 1 per cent.	1 to 5 per ct.	6 to 10 per ct.	11 to 15 per ct.	16 to 20 per ct.	Average per ct.
Tubes standing 24 hrs.	4	6*	0	0	1*	+2
" " 48 "	0	8	4	0	0	−5

* One case under each starred column denotes a gain; all others indicate a loss of percentage given as compared with the first determination.

The longer settling period without disturbance results thus in a slightly diminished volume, but, as comparison with previous tables shows, the diminution is much less than if the tubes had been disturbed during the settling.

This was further illustrated by the effect of the location on volume; such tubes as were left on a table subject to vibration settled more compactly than the same hauls left an equal time in a position free from vibration.

The size of the settling tube has also a marked influence upon the amount obtained. Kofoid (97, p. 19) mentions the use for small planktons of a tube 6 mm. in inside diameter whereas those in ordinary use were 10 mm. in inside diameter. In the first measurements we used tubes of two diameters, 8 mm. and 10 mm., until remeasurements of the planktons originally tried in the smaller tubes showed a loss of 30 per cent., whereupon larger tubes, about 15 mm. in diameter, were also used for comparison. Four planktons were measured in the ordinary tubes and then in 15 minutes remeasured; the loss was 3, 4, 2, 3 per cent. respectively. The same planktons were then poured into a broad tube and after 21 days the volumes measured differed from these first taken in the ordinary tubes by -9, 0, −2 and 1 per cent.; in 22 days the figures stood -14, -4, -11, -14 per cent., and in 55 days -23, -11, -19, -20 per cent. Of another four two were left in the same tubes and two others changed to broad tubes with the following results:

Plankton measured after 24 hours	Loss in 15 minutes	The plankton was then	Loss per cent. after 21 days	22 days	43 days	55 days
3.90 cc.	3 per cent.	Kept in same tube	4	9	11	
8.55 cc.	3 per cent.		6	7	8	
9.32 cc.	6 per cent.	Transferred to large tube	1	7	13	19
9.70 cc.	5 per cent.		2	10	20	27

These data show again that the reduction in volume is consequent upon handling or disturbance rather than upon time of settling and apparently also that the loss in volume was somewhat greater in the larger tubes, other conditions being apparently identical.

Regarding the personal equation in such measurements, the following data are given: A total of 50 hauls was measured by Miss Fossler and myself independently and within a short time of each other. The average variation was 3.6 per cent.; a variation of more than 20 per cent. occurred in 2 cases, of 10-20 per cent. in 20 cases, of 5-10 per cent. in 11 cases, of less than 5 per cent. in 17 cases, in three of which the amounts obtained were actually identical. These results may now be compared with those obtained at widely separate time intervals by the same person. For such comparison in the gravimetric method three sets of figures, made from the same hauls at intervals of a year and a half, are given in the original and first and second supplementary sets, and their comparison yields the following table:

Compared with the first	Gain 10 to 15 per ct.	Gain 5 to 10 per ct.	Gain 1 to 5 per ct.	Same	Loss 1 to 5 per ct.	Loss 5 to 10 per ct.	Loss 10 to 15 per ct.	Loss 15 to 20 per ct.
The second measurement shows the following cases	3	4	7	5	21	27	19	9
The third measurement shows	2*	9	13	8	27	17	12	6
Total cases	5	13	20	13	48	44	31	15

These figures seem to indicate a slight average reduction in amount with time and yet the third series was measurably larger than the second though made a year and a half later.

* One case 60 per cent. gain was evidently due to an error in reading or recording.

When it is considered that out of 196 cases in all, 81 or nearly half vary less than 5 per cent. either way in three successive measurements and 57 more fall within the 10 per cent. limit, leaving a total of 58 or between one-third and one-fourth all of which however come within the 20 per cent. limit, it may fairly be claimed in view of the actual variations in the plankton itself that the results obtained in this way by the gravimetric method are comparatively uniform for the same haul.

If however different hauls, and especially those containing different kinds of plankton organisms, be compared, the results are otherwise. On this point Kofoid says (97, p. 19): "Planktons do not settle to an equal density. Those composed of Rotifera or small Cladocera (as *Chydorus*) pack closely, while others containing filamentous forms, as *Oscillaria* or *Fragilaria*, and those in which the larger Entomostraca are predominant settle very loosely. Thus the determination of the volume of the plankton by the settling method does not give a uniform test of the amount of plankton present. Furthermore the process is a tedious one, especially when large numbers of catches are to be handled."

With the latter statement, every one who has employed the gravimetric method on a considerable series of hauls will most heartily agree. The former is true whenever the hauls are commingled with any of the filamentous algae, but when these are absent it is only true within limits which are in fact less than the limits of normal variations in the amount of plankton.

These points are still clearer from an examination of the plates where the position of the lines indicates the relation of the various series of measurements. The fact that the three lines indicating the successive sets of gravimetric measurements cross and recross diverging and approaching indicates that one set of measurements does not show a constant loss or gain as compared with any other, such as might be due to gradual shrinkage of volume with time, or to any other single factor; but rather that the measurements vary indefinitely from one another.

Regarding gravimetric measurements in general it may be stated further that at least two factors, the diameter of the settling tube and the length of time during which the tube is left standing are entirely arbitrary. The former influences the volume considerably, the latter somewhat though not so noticeably as some external factors.

If now the results obtained by the gravity method and by the use of the centrifuge be compared with each other, as may be most easily done from the graphic presentation of results given in the plates, it appears first, as could have been predicted, that the amounts obtained by the centrifuge are constantly less than those measured in settling tubes. This is not only true of the amounts obtained by direct measurement (Pl. XV) but also of those representing the stratal volumes which are obtained indirectly. So generally is this the case that a few observations, such as XVIII (2, Pl. XVII) where one gravimetric value departs so widely from others obtained by the same method as to fall far below those measured in the centrifuge, impress one as distinctly erroneous and in all probability attributable to errors of notation. The amounts obtained by centrifuge measurement range from 30 to 40 per cent. of those recorded from the settling tubes for the same haul; the average being slightly more than one-third; the results differ, however, according as one series or the other be taken on either side as the basis of comparison. Juday (97) found that the centrifuge gave about one-fifth of the volume obtained by settling. His method varied in detail from that which we used, employing 100 revolutions of the crank in centrifuge measurements; no details are given regarding the precise method of gravity measurements.

In the next place it will be noted that the lines indicating the centrifuge measurements follow regularly those of the gravity measurements with but a single marked exception, that already noted as probably an error (Haul XVIII, 2, Pl. XVII); but that in general the variations of the centrifuge line are less violent. This is beautifully shown in a comparison of the lines

of total volumes (GT and CT, Pl. XV). For the sake of distinctness the latter were dropped 7 ccm. and were measured from the double line as a base. They stand thus everywhere clear of the lines (GT) portraying the results of the gravimetric method and while following in general the movements of the latter they do so everywhere with less intensity. The same appears from a comparison of the lines of average volume (GA and CA) on the same plate.

When the three sets of centrifuge measurements are compared to one another using the first as a basis, the following figures are obtained:

Compared to first	Gain of								Loss of						
	35 to 0 per ct.	30 to 25 per ct.	25 to 20 per ct.	20 to 15 per ct.	15 to 10 per ct.	10 to 5 per ct.	5 to 1 per ct.	No difference	1 to 5 per ct.	5 to 10 per ct.	10 to 15 per ct.	15 to 20 per ct.	20 to 25 per ct.	25 to 30 per ct.	30 to 35 per ct.
No. cases in second measurement...	4	3	4	4	16	18	18	2	10	5	9	1	3	1	0
No. cases in third measurement...	2	2	9	1	10	11	17	2	10	14	10	3	5	0	2

The average advance in the hauls showing a gain is 11.82 and 11.63 per cent., and the average reduction in hauls showing a loss is 10.62 and 11.41 per cent., results which are strikingly uniform.

If these results be compared with those obtained in the three gravity measurements, the first series by each method being taken as a basis for the computation of percentages in other series by that method, the methods show the following differences in the later series of measurements.

	Gravity measurement	Centrifuge measurement
Less than 5 per cent. difference, either more or less	84 cases	59 cases
From 5 to 10 per cent. difference, either more or less	57 "	48 "
More than 10 per cent. difference, either more or less	51 "	89 "
Total number	192 cases	196 cases

The decidedly better showing on the part of the older gravity method is certainly due in part to the smaller quantities obtained by centrifuge measurement and the consequent greater percentage of the whole which a minute difference constitutes. In actual amounts the centrifuge measurements were very close as shown by the following comparison of the second and third sets with the first.

				Second	Third
No. cases from	0.00	to	0.05 ccm. less or greater than in first,	27	34
" " "	0.05	to	0.1 " " " " " " "	33	23
" " "	0.1	to	0.2 " " " " " " "	15	21
" " "	0.2	to	0.2 " " " " " " "	9	11
" " "	0.3	to	0.4 " " " " " " "	3	4
" " "	0.4	to	0.5 " " " " " " "	7	2
" " "	0.5	to	0.6 " " " " " " "	0	0
" " "	0.6	to	0.7 " " " " " " "	1	2

Here 67 cases show a greater amount in the second measurement than in the first and only 26 a less; the measurements were made by different individuals, indicating a distinct though slight tendency on the part of the second experimenter to increase volumes either in reading amounts in the centrifuge tubes or in unconsciously modifying the process so as to secure a larger amount in fact through less condensation.

In the third the number of cases showing a greater amount than the first was only 54, a notable reduction from the condition in the second set of measurements, although the second and third sets were both made by the same individual. There is thus evident a slight variation in the results obtained by the same person at different times.

A general comparison of the value and utility of the two methods of measurements suggests the following. In settling

tubes a uniform density is certainly not obtained, for as both Kofoid and I have noted, different plankton hauls settle so unevenly as to present to the eye regions of variable density in the graduated tube while in the centrifuge tubes no difference is apparent. The centrifuge affords certainly a more speedy and more easily manipulated method, one that does not depend at all so far as observed on environment, i. e., external vibration, handling of tubes, etc., and one which furthermore is not so liable to accident as in the case of long settling tubes standing full for twenty-four hours. Owing to the peculiar conical tip of the centrifuge tube a small quantity may be more precisely estimated than one which is larger and hence is open to less percentage of error than if measured in a settling tube of approximately equal calibre throughout. Finally it is indisputable that with the centrifuge conditions may be much more precisely stated in measurable terms, and consequently repeated by other observers with a greater chance of obtaining similar conditions and hence results directly comparable. In fact I may confess that I entered upon the final comparison with a distinct prejudice in favor of the centrifuge so strong that it has not been entirely removed by the apparently negative results of this series of observations, where, as noted above, the centrifuge measurements are not so close in percentage of volume as those made by the gravity method.

One recent writer (Fuhrmann, 99) refers to the centrifuge method as detrimental to the plankton if desired for future study. This is certainly not the case with the hauls used in these experiments; after six years' time and all the manipulation noted the various planktonts are apparently as good for microscopic study or for numerical estimation as they were at the start. Dr. Kofoid informs me in correspondence that his experience with plankton hauls measured in the centrifuge has been the same as mine.

Some experiments were conducted with the centrifuge to determine the influence of time of rotation on amount obtained. One dozen hauls, measured in one-half minute with 40 revolu-

tions of the crank, i. e., half the usual time and number of revolutions, yielded results which in 4 cases were less and in 7 cases more than the figures obtained in the regular series. The kind of plankton made so far as could be seen no difference in results and the size of tube used in measuring could not be varied in the experiments so that I am unable to say how, if at all, it influenced the results.

Finally one may inquire as to the question of paramount importance, how all the variations in each method and how the change of method would affect the general statements previously made regarding the distribution and relative mass of plankton in the various strata in Lake Michigan. In the original paper (Ward, 96b) it was said:

"1. The total volume increases with the depth but more rapidly for depths up to about 30 meters than beyond that point." This is illustrated in the lines CT and GD, Plate XV.

"2. The volume per cubic meter of water decreases as the water grows deeper. This decrease is irregular for shallower stations, but comparatively constant in deeper water." This is shown by the lines GA and CA, Plate XV.

"It is at once apparent that the surface stratum contains a much greater quantity than any other stratum, on the average more than twice as much, while the intermediate strata are not far from equal. * * * The line of volumes in the surface stratum, 0 to 2 meters (Plate XVI, B), pursues a somewhat irregular course. The irregularities are independent of the depth and of the total and relative volumes of plankton. * * The five to ten meter stratum contains more plankton per cubic meter of water than the two to five meter stratum."

The results of the six series of measurements given on the charts unmistakably show that whichever method or series be taken all statements made regarding the amount and distribution of the plankton hold good. All variations and errors are not enough to obscure or render doubtful the general relations between the amounts of plankton found in the various strata.

Of errors common to both methods there may be noted two: that of estimation, and that of notation, as they may be called.

Variations in measurements are thus sometimes brought about by the liability to personal error to which both methods are open. The upper surface of the plankton mass is uneven both in the settling tubes and in the centrifuge, and the estimation of the actual value involves a chance of error which is proportionately greater as the amount measured is less. It is often very difficult to estimate the mass, as when the upper surface is hollowed out. What the limits of this error may be I can not show, but they are probably narrow.

To this must be added the well known errors due to the use of figures. These are made in reading the scale and in recording the amounts. That such do occur, all who have tried either method are convinced and think that in them may be found the cause of isolated values far removed from those of the same haul in the other two or more sets of measurements. Such a case is apparently Haul XVIII (Pl. XVII) in which two values obtained by the gravity method are positive and in practical agreement, the third by the same method is negative and evidently imaginary.

In the light of the general close correspondence in the volume of a haul at different measurements by the same method, a difference of 60, or even of 37 per cent. as noted in two isolated cases can hardly be other than a mistake in reading or copying the amount observed. Each assistant has independently made mention of the likelihood of a slip of this character and of the difficulty of guarding against it entirely.

It should not be forgotten that there may be differences due to the actual loss of a quantity of material from a haul at one stage or another during the long drawn out period of manipulation. At the start there was no intention of using these records for comparative purposes, and losses, if any, occurring after a measurement was completed, were not recorded as they did not affect the series just made and other series were not planned until later. Such a hypothesis will match well one or two radical differences, shown on the plates, but in the absence of positive evidence it is enough to have referred to the possibility.

The fourth question propounded at the start: What is the error in the enumeration or estimation of the individuals in the volume obtained, may be only briefly touched upon as it was not included in the series of experiments which form the basis of this paper. Personally I feel that a mere tabulation of the number of individuals of given species in a given haul is of little value; it certainly misleads one as the different individuals are of enormously different quantitative value. Whipple (94b) has brought forward a method which does away to a large extent with the misconception aroused in that these values are expressed in terms of a given unit area. This is undoubtedly very helpful in many cases in arriviug at a just idea of the relative importance of different species. On the whole, however, I am inclined to think that the general productivity of a lake will be expressed in the form of volumes as is the case with land surfaces. It would add nothing to our conception of the fertility of a field to state it in number of kernels rather than in bushels of corn or wheat; similarly the economic measure of a water basin will not be modified by the millions of bacteria it contains however much it may be dependant upon the same in ultimate biologic analysis. To this extent, then, I think the statements of Kofoid are apt to be misleading when he refers to the enormous number of minute planktonts which escape the meshes of the net. They are unquestionably important, their number should be determined and their part in the economy of the water clearly fixed, but they are still insignificant from a general standpoint.

The question of the extent to which enumeration should be carried, and above all that of the actual meaning of the results obtained needs careful elucidation. On this matter I have no data to offer here.

Field (98) maintains that volumetric estimation is indispensable and has made use of a large form of the centrifuge in the precipitation and measurement of living oceanic plankton. This form, known as the planktonokrit, was originally described by Dolley (96). While Field holds that the centrifuge is a rapid and accurate means of determining the plankton volume

he does not refer to the extent or character of the evidence on which such a view is based, and in a previous paper (Field, 97, p. 425) states that certain forms are packed more closely than others by centrifugal force, which would indicate another source of error in comparative estimations.

CONCLUSIONS.

1. Some part of the plankton is not caught in the vertical net since a) the latter does not filter the entire water column, and b) some organisms pass through its meshes. Neither factor has yet been precisionated. The amount and constancy or variability of this error should be precisely determined.

2. In manipulations incident to preservation the loss of plankton is insignificant.

3. In measuring plankton by the gravimetric method, the age of the preserved material is immaterial; the amount of disturbance to which the plankton tubes are subjected during settling is much more important in modifying the volume than the length of time they stand, and the diameter of the settling tubes influences strongly the results obtained.

4. Under similar conditions the results obtained by the gravimetric method are comparatively uniform for the same haul, but vary with the kind of plankton measured.

5. Measurements made with the centrifuge yielded volumes from one-third to two-fifths as large as those obtained by the gravimetric method.

6. In the series of measurements made, the centrifuge method showed greater variation than the gravimetric.

7. Both methods agree as to results in all essential points and no series of measurements vitiated or modified the statements regarding the general distribution of plankton in Lake Michigan as originally deduced from the hauls.

8. The centrifuge method appears to have greater general utility, and uniformity sufficient to call for its preference; with plankton hauls differing radically in composition it would probably be more uniform than the gravimetric.

9. It does not injure the most delicate material obtained.

10. Errors of estimation, of notation, and those due to actual loss, are likely to occur in any series of measurements.

11. Evaluation by volume furnishes more usable values than mere numerical estimation. The latter may be improved by areal estimation.

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EXPLANATION OF PLATES.

Vertical lines indicate stations, each of which is designated by a Roman numeral at the upper end.

Horizontal lines represent volumes or depths, each square indicating ten meters in depth or one cubic centimeter in volume, the total being reckoned from the upper margin of the chart, except for CT (q. v.).

D....D indicates depth of the various stations.

GT....GT represents total volume of plankton measured by the gravimetric method.

CT....CT indicates total volume of plankton measured by the centrifuge. The volume is here reckoned from the double line near the middle of the chart as a base rather than as all others from the top line of the chart.

GA....GA represents estimated volume of plankton per cubic meter of water measured by gravimetric method.

CA ...CA represents estimated volume of plankton per cubic meter of water measured by centrifuge.

0. Original set of measurements, made by gravimetric method.
1. First supplementary set by gravimetric method.
2. Second supplementary set by gravimetric method.
3. First set of measurements made by centrifuge.
4. Second set of measurements made by centrifuge.
5. Third set of measurements made by centrifuge.

For further details see text; also Ward, 96, 96b.

PLATE XV.

A GRAPHIC REPRESENTATION OF AMOUNTS IN BOTTOM HAULS FROM LAKE MICHIGAN MADE WITH VERTICAL NET.

PLATE XVI.

A GRAPHIC REPRESENTATION OF STRATAL HAULS.

A. Stratum 25 meters to 10 meters.
B. Stratum 2 meters to surface.

PLATE XVII.

A GRAPHIC REPRESENTATION OF STRATAL HAULS.

A. Stratum 5 meters to 2 meters.
B. Stratum 10 meters to 5 meters.

PLATE XV

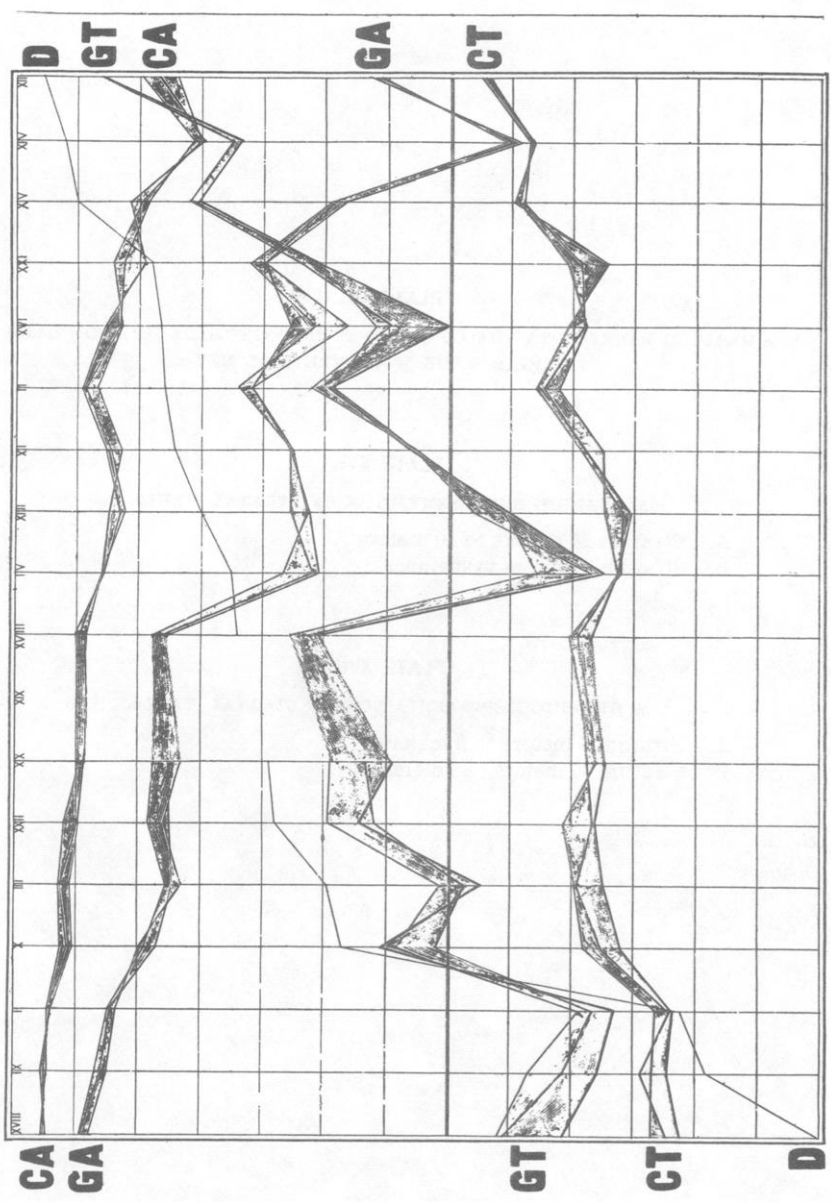


PLATE XVI

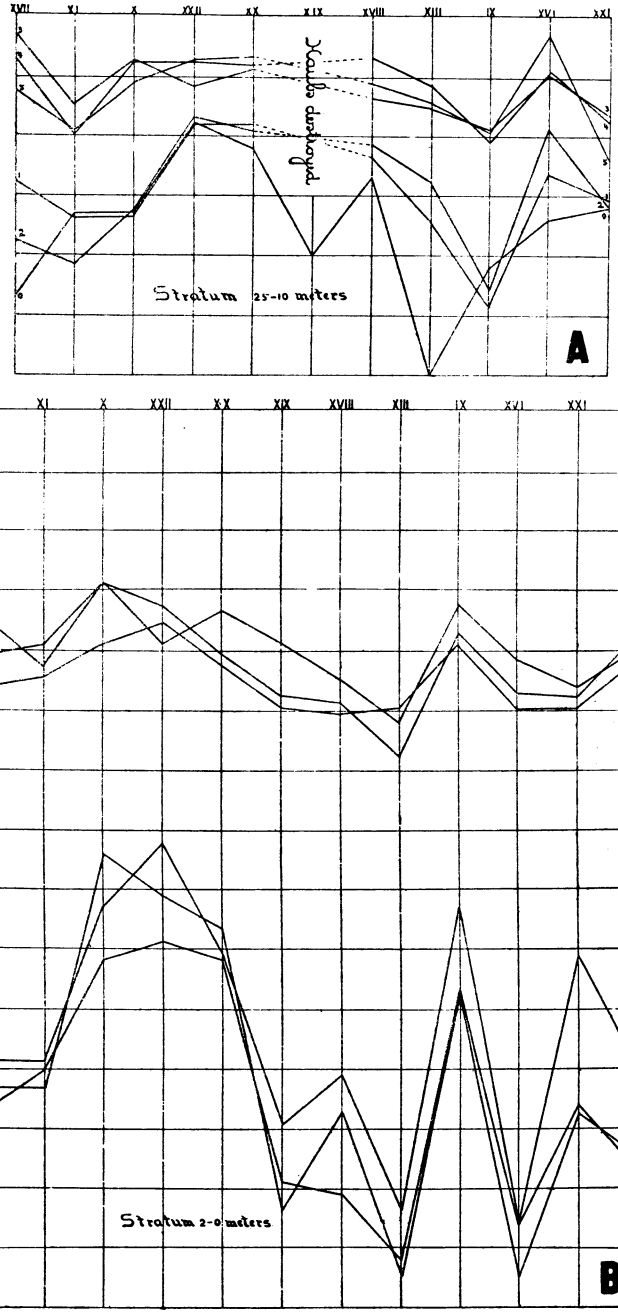
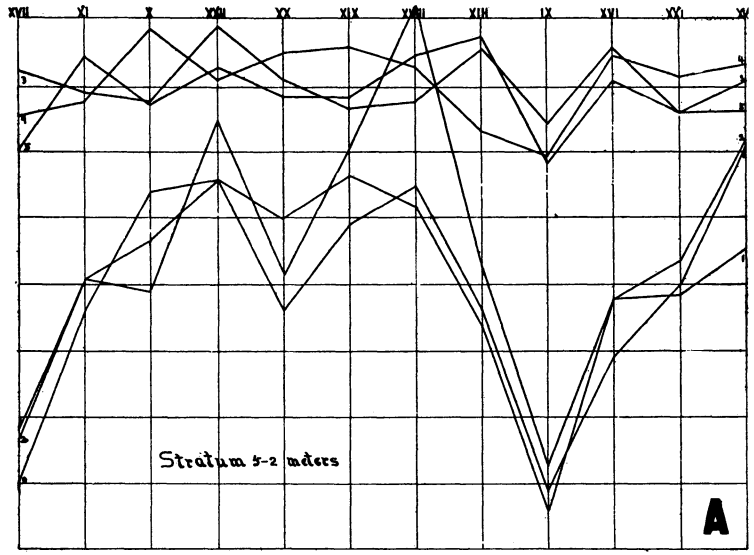
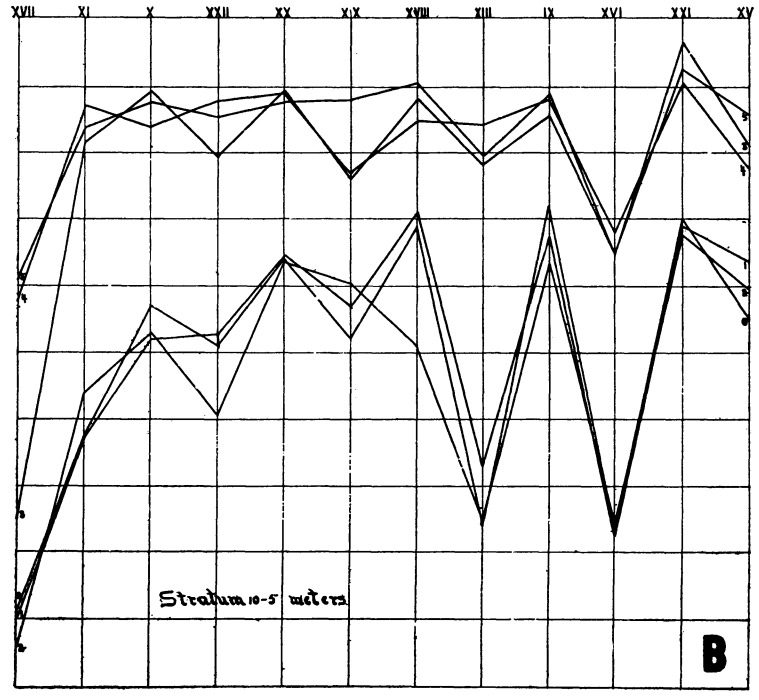

XVII XI X XXII XX XIX XVIII XIII IX XVI XXI
Kaulb dunkoyd
Stratum 25-10 meters
A
XVII XI X XXII XX XIX XVIII XIII IX XVI XXI XV
Stratum 2-0 meters
B

PLATE XVII


XVII XI X XVII XX XIX XVIII XIII IX XVI XXI XV
Stratum 5-2 meters
A


XVII XI X XVII XX XIX XVIII XIII IX XVI XXI XV
Stratum 10-5 meters
B